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A New Dosimetric System Based on Aqueous Alcoholic Solutions of Tetrazolium Salt

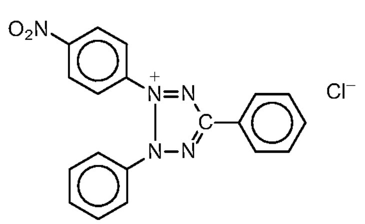
Alexei K. Pikaev* and Zoya K. Kriminskaya

Institute of Physical Chemistry, Russian Academy of Sciences, 117915 Moscow, Russian Federation. Fax: +7 095 335 1778

A highly-sensitive dosimetric system based on aqueous alcoholic solutions of tetrazolium salt has been developed: an aerated 0.4–1×10^{-2} mol dm^{-3} aqueous alcoholic (isopropyl alcohol or ethanol, volume ratio water:alcohol = 1:1) solution of 2-(*p*-nitrophenyl)-3,5-diphenyltetrazolium chloride containing 0.2 mol dm^{-3} sodium formate (pH 9.5–11). The system can be used for the determination of γ-radiation doses within the range 1–5 to 50 Gy.

Data exist in the literature on the possible use of solutions of tetrazolium salts in the dosimetry of ionizing radiation. According to ref. 1, a solution of 2,3,5-triphenyltetrazolium chloride (TTC) in water (or in water containing 2% agar) can be used for the determination of doses within the range 0.8–120 kGy. It follows from ref. 2 that a 4.5 × 10^{-2} mol dm^{-3} solution of this compound in ethanol allows measurement of doses from 1–16 kGy. The action of these systems is based on the radiolytic reduction of colourless tetrazolium salt to an intensely-coloured (red or orange red) formazan [λ_{max} = 480–485 nm, ε_{max} = (1.1–1.7)×10^3 m^2 mol^{-1}]. However, the sensitivity of the systems under consideration is limited by the presence of oxygen from the air in the solution. Oxygen, reacting with solvated electrons, H atoms and radicals from the alcohol, forms hydroperoxide or peroxide radicals which are unable to reduce TTC and/or interact with the semireduced form of the salt (*i.e.* tetrazolium radical), preventing the formation of formazan.[3–7]

On studying the influence of the nature of tetrazolium salt on the mechanism of its radiolytic conversion it was deduced that the oxygen effect is absent for solutions of 2-(*p*-nitrophenyl)-3,5-diphenyltetrazolium chloride (NTC).[7]



NTC

Apparently, the use of this compound can lead to a considerable increase in the sensitivity of the dosimetric system.

Two cobalt-60 facilities were utilized as sources of γ-radiation. These allowed us to carry out the experiments using dose rates from 0.033–11 Gy s^{-1}. All the reagents were of analytical grade; additional purification was not required. Since formazan (a product of the reduction of tetrazolium salt) is poorly soluble in water, aqueous alcoholic solutions were used; in general, aqueous isopropyl alcohol solutions of NTC (with volume water:isopropyl alcohol ratio = 1:1) were utilized. For comparison, aqueous solutions of tetrazolium salts were also studied. In this case the precipitate of formazan formed was dissolved by the addition of isopropyl alcohol–water (1:1). In several cases the radiolysis of deaerated solutions was performed. The removal of air was achieved by bubbling purified argon through the solutions during 40 min. A 'Specord UV Vis' spectrophotometer was used for the measurement of the optical absorption spectra of initial and irradiated solutions. Molar extinction coefficients at λ_{max} for formazans formed upon the reduction of TTC and NTC (*i.e.* at 485 and 475–480 nm), were determined to be equal to 1.6×10^3 and 1.9×10^3 m^2 mol^{-1}, respectively.

It was confirmed that oxygen from the air present in an aqueous solution of TTC has a considerable effect on the formazan yield. This is shown in Fig. 1, in which the dependence of the formazan concentration, formed upon γ-irradiation of deaerated and aerated 1×10^{-3} mol dm^{-3} aqueous solutions of TTC, on dose are represented. It is seen that in aerated solution the formation of formazan at doses <800 Gy occurs in extremely low yield; at doses >800 Gy, when oxygen was consumed, the yield increases considerably. In contrast, the influence of oxygen on the formation of

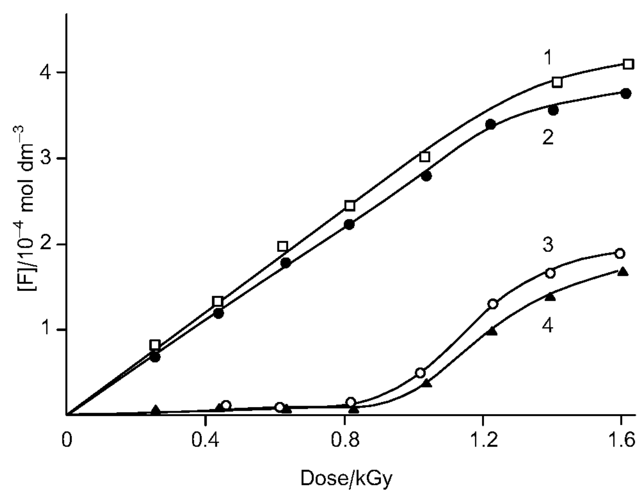


Fig. 1 The dependence of the concentration of formazan formed in a 1×10^{-3} mol dm^{-3} aqueous solution of 2,3,5-triphenyltetrazolium chloride on dose: 1, deaerated solution, pH 10.3; 2, deaerated solution, pH 8.5; 3, aerated solution, pH 10.3; 4, aerated solution, pH 8.5.

formazan upon radiolysis of neutral aqueous solutions of NTC is comparatively small; in alkaline solutions the effect is completely absent.

The reduction of tetrazolium salts to formazans is a bielectronic process. In aqueous solutions it occurs in reactions with hydrated electrons e^-_{aq} and H atoms. In aerated solutions of TTC the tetrazolium radicals, formed as a result of these reactions, react with oxygen giving the initial tetrazolium salt. In addition, e^-_{aq} and H partially interact with oxygen, forming HO_2 and O_2^-, which do not reduce TTC. In the case of NTC the reaction of tetrazolium radicals with oxygen does not occur; in addition, NTC is reduced by radical ions O_2^-. These reasons explain the absence of the influence of oxygen on the formation of formazan in alkaline media.[7]

The addition of alcohol to slightly alkaline aqueous solutions of NTC gives rise to a considerable increase in the radiation chemical yield of formazan (see Table 1). This is due to the participation of radicals from the alcohol (products of the reaction between OH radicals and alcohol and of alcohol radiolysis) in the reduction of NTC. However, in this case the reaction of radicals from alcohol with oxygen is possible; peroxide radicals formed in this reaction seem to be incapable of reducing NTC. Therefore, it is necessary to use a concentration of NTC as high as possible so that radicals from the alcohol react predominantly with tetrazolium salt. Thus, we studied aerated 4×10^{-3} mol dm^{-3} solutions of NTC in a mixture of water–isopropyl alcohol (1:1) at pH 9.5–11. Note that at higher pH values the stability of NTC solutions upon storage decreases noticeably (spontaneous coloration of the solutions occurs after several hours).

Fig. 2 (curve 1) shows the dependence of the optical density of a 4×10^{-3} mol dm^{-3} aqueous isopropyl alcohol (1:1) solution of NTC (pH 10.5) at λ = 475 nm (the peak of the optical absorption band of formazan formed) on the dose of γ-radiation. It is seen that this dependence is linear within the dose range 10–50 Gy. At lower doses an 'induction' period is observed; at doses over 50 Gy there is a deviation from linearity (because of the reduction of formazan itself).

It is possible that the 'induction' period is connected with the partial interaction of radicals from the alcohol and the formation of peroxide alcoholic radicals which are unable to reduce NTC. It was found that this period is absent if 0.2 mol dm^{-3} sodium formate was added to the solution (see Fig. 2, curve 2). In this case the linear dependence of optical density on dose takes place in the dose range 5–50 Gy. A possible reason for the removal of the 'induction' period is the reaction of radicals from the alcohol with formate and their conversion to radical ions COO^- which have a higher reactivity towards NTC. The sensitivity of the system

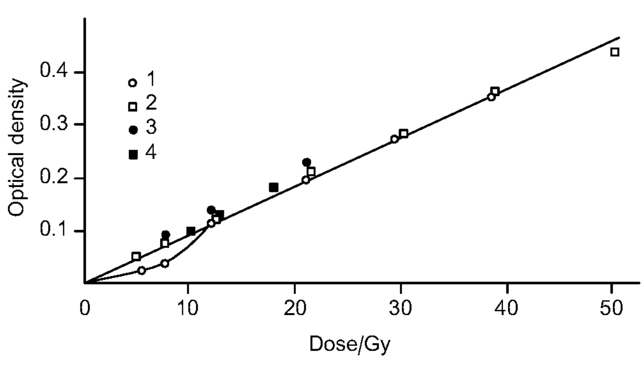


Fig. 2 The dependence of optical density (λ = 475 nm) of aerated aqueous alcoholic (volume ratio = 1:1) solutions of NTC on dose: 1, 4×10^{-3} mol dm^{-3} aqueous isopropyl alcohol solution, pH 10.5; 2, the same solution, containing 0.2 mol dm^{-3} sodium formate; 3, 1×10^{-3} mol dm^{-3} aqueous isopropyl alcohol solution containing 0.2 mol dm^{-3} sodium formate, pH 10.5; 4, 4×10^{-3} mol dm^{-3} aqueous ethanolic solution containing 0.2 mol dm^{-3} sodium formate, pH 9.5. Optical path length: 1 cm.

Table 1 Radiation chemical yields of formazan G(F) upon γ-radiolysis of aqueous and aqueous isopropyl alcohol (1:1) solutions of TTC and NTC.

Compound	Concent-ration/ mol dm^{-3}	Presence of alcohol	Gas satura-ting the solution	pH	G(F), molecules/ 100 eV
TTC	8×10^{-4}	Yes	Argon	11.0	3.1
	8×10^{-4}	Yes	Air	11.0	0.14
	1×10^{-3}	No	Argon	6.9	0.46
	1×10^{-3}	No	Air	10.3	1.2
NTC	1×10^{-3}	No	Argon	6.9	0.6
	1×10^{-3}	No	Air	6.9	0.45
	1×10^{-3}	No	Argon	11.3	1.3
	1×10^{-3}	No	Air	11.3	1.5
	4×10^{-3}	Yes	Air	10.5	4.9
	1×10^{-2}	Yes	Air	10.5	5.3

developed upon spectrophotometric measurements is equal to 6×10^{-3} Gy^{-1} cm^{-1}. If a 1×10^{-2} mol dm^{-3} solution of NTC is used, the sensitivity increases to 1×10^{-2} Gy^{-1} cm^{-1} (see Fig. 2), and the threshold of the measured dose decreases to 1 Gy.

The irradiated solution is sufficiently stable upon storage. The optical density of the solution after standing in the dark during 24 h changes by not more than 3%. The confidence interval for the reproducibility of measurements determined with reliability 0.95 is not more than 30% at dose 5 Gy and 10% at dose 50 Gy.

It was found that changing isopropyl alcohol for ethanol has no effect on the sensitivity of the system (see Fig. 2). The sensitivity of an aerated 4×10^{-3} mol dm^{-3} aqueous ethanolic (1:1) solution of NTC (pH 9.5) is 6×10^{-3} Gy^{-1} cm^{-1}.

The system developed here can be recommended for the dosimetry of ionizing radiation in radiation processing (*e.g.* in radiation treatment of food), in some areas of radiation chemistry and in radiobiology.

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